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## Identification of a secondary Fc gamma RI binding site within a genetically engineered human IgG antibody.

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Although human IgG2 is not cytophilic, we have shown previously that an IgG2 antibody expressing the sequence PLLGG (underline = substitution) spanning CH2 domain residues 233-237 (Eu numbering) displayed IgG1-like Fc gamma RI binding activity. In contrast, IgG1 PLLGG exhibited 3-fold less affinity, whereas IgG2 ELLGG was 3-fold more active than native IgG1. These results suggested that additional site(s) conferred enhanced binding properties to the engineered, cytophilic IgG2 variant. These sites were shown to reside in the IgG2 CH2 domain, since the IgG1 CH2 module did not have enhanced activity in a panel of hybrid IgG1/IgG2 antibodies. To map these sites further, human IgG1 and IgG2 constant region gene segments were modified to allow reciprocal COOH-terminal half segment exchanges of CH2 exons. These were cloned into a pSV2neo expression vector bearing a rearranged MOPC 315 heavy chain variable region gene and transfected into a MOPC 315 heavy chain deletion mutant. The dinitrophenol affinity-purified IgGs were radiolabeled and assessed for Fc gamma RI binding activity in direct binding assays using U937 cells. The COOH terminus of the IgG2 CH2 domain was found to contain accessory site(s) since it enhanced the binding properties of both IgG1 PLLGG and native IgG1. In contrast, grafting of the COOH terminus of the IgG1 CH2 domain onto IgG2 PLLGG and IgG2 ELLGG diminished their cytophilic activity. The amino acid responsible for the enhancing properties of the COOH terminus of the IgG2 CH2 domain was shown to be threonine 339, since IgG1 PLLGG/Thr339 displayed increased Fc gamma RI binding affinity. Kinetics studies revealed that this is accomplished through an increase in the forward rate constant of the IgG-Fc gamma RI interaction.

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